DIFFERENTIAL SCANNING CALORIMETRY OF NONFREEZABLE WATER IN SOLUTE–MACROMOLECULE–WATER SYSTEMS

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ABSTRACT

Differential scanning calorimetry was employed to determine the amount of bound (nonfreezable) water in several model systems as a function of water activity (a_w). Water activity was controlled by varying total moisture content or by adding a solute, urea, to the aqueous phase of the model system. Since the amount of bound water is dependent on the nature of the components, correlations between bound water and a_w are meaningful only for specific systems. In every case studied, bound water, as g H_2O/g solids, decreased with decreasing a_w, along what might be called a bound water isotherm. The results indicate that measurements of bound water should refer to a specified value of a_w. In addition, a_w of the solution phase appears to be a major contributor to the driving force for water binding by macromolecules.

INTRODUCTION

THE MOISTURE CONTENT of foods is frequently described as consisting of two portions: bound and free. Although the definitions of these two states of water are not universally accepted and subdivisions of these states are possible, one usually construes bound water to mean that portion of the total moisture which has measurable properties that differ from those of bulk water or simple solutions (Kuntz and Kauzmann, 1974). These differences may be attributed to interaction forces between the water molecules and the various "solid" components of the food. Hence, the classification bound water refers to surface moisture and the first few layers surrounding the solids.

Just as the total equilibrium moisture content of a food varies with water activity (a_w) according to the moisture sorption isotherm, one may presume that the bound water content should also vary with a_w. Several studies attempting to correlate bound water with a_w have been reported (Karmas and Chen, 1975; Leung et al., 1976; Bechtel et al., 1971), but the search has tended to concentrate on a universal correlation for all systems, regardless of the types of components. In this manner, one would hope to measure only the amount of bound water to specify the a_w of the system. A universal correlation has not been attained, however, so specific systems of fixed composition should be investigated.

The objective of the present work was to measure bound water by a currently accepted method over a range of a_w in several model systems. In each model system, a_w was controlled by adjusting total moisture or by adjusting the concentration of one of the soluble components. Thus, the confounding effects of inherent differences in water binding between individual components was minimized.

EXPERIMENTAL

BOUND WATER was determined by differential scanning calorimetry (DSC) as the amount of nonfreezable water within a sample after being cooled to at least −70°C (Simatos et al., 1975) with liquid nitrogen. When the temperature of the cooled samples is increased at a constant rate in the calorimeter, the fusion of ice is detected as an endothermic peak with area proportional to the amount of ice present. Nonfreezable water is simply the difference between total water content and the amount of water detected by the fusion endotherm. Cooling to any temperature between −70 and −100°C gave no difference in results. Samples of 10–15 mg were encapsulated in hermetic aluminum pans prior to measurement with a DuPont Model 990 Thermal Analyzer at a heating rate of 1°C min^{-1}. Enthalpy determinations were made by measuring fusion peak areas with a polar planimeter. Since samples containing urea typically gave two fusion endotherms (see below), the areas of each individual peak were obtained after extrapolating the initial slope on the high temperature side of the eutectic peak back to the baseline. A standard curve of relative peak areas versus concentration was constructed. Pure H_2O was used to calibrate both temperature and fusion enthalpy.

Water activity was determined by one or more of three methods: 1. Electric hygrometer (Hygrodynamics, Inc., Silver Spring, MD) 2. Dew point hygrometer (EG&G International, Inc., Waltham, MA) 3. Calculation on the basis of component concentrations (Ross, 1975).

Two model systems, IMF 1 and IMF 2, were prepared as "dry" mixes to which varying amounts of water were added to obtain appropriate levels for total moisture and a_w. IMF 1, one of the model intermediate moisture foods of Karmas and Chen (1975), contained corn oil, carboxymethyl cellulose, Na-caseinate and glycerol in a weight ratio 1:12:12:25. Sufficient water was added to give samples with total moisture in the range 28.6–80.0% on a wet basis. IMF 2 contained ground beef, soy flour, sucrose, NaCl and lard (11:13:8:1.5:1:1) at moisture levels between 29.5 and 47.5% on a wet basis. Total moistures in IMF 1 were calculated from the moisture contents of the various components plus the weight of pure water added. Karl Fischer titrations were performed on IMF 2 samples because of the high moisture levels in ground beef.

Na-caseinate was dispersed in pure water at several concentrations to provide a third system for study. Moisture levels were determined from the weight of water added to the caseinate, as well as by drying the individual DSC sample pans in a vacuum oven after puncturing the lids with a pin.

The Na-caseinate was fractionated into high- and low-molecular weight components by dialysis. A 15% dispersion of caseinate in water was dialyzed by a negative pressure technique (Fox et al., 1967) to obtain the low-molecular weight species. These dialyzable materials were lyophilized and then redispersed in pure water at several concentrations for further study. The caseinate was also exhaustively dialyzed against pure water, which was changed at least twice daily for 1 wk (a drop of toluene was added to the water to inhibit microbial spoilage). After 1 wk the casein was milky white in color and had precipitated to the bottom of the dialysis tubing, indicating that the removal of solutes was essentially complete. This isoelectric casein was sampled directly from the dialysis tubing for DSC experiments at 5, 10 and 15% total solids. The remaining isoelectric casein was lyophilized for further sample preparation.

Another model system was prepared by redispersing isoelectric casein in urea solutions of concentration 0.5–6.0 molal, such that the ratio of casein to water was constant at about 1:9 by weight.

A fifth model system was prepared by dispersing Avicel microcrystalline cellulose (FMC Corp., Newark, DE) in solutions of urea. The Avicel to water ratio was fixed at about 1:6 by weight.

RESULTS & DISCUSSION

THE RESULTS for model IMF 1 are graphically displayed in Figure 1. Nearly all moisture is bound at a_w 0.7 and below, i.e., the water fusion endotherm is vanishingly small below a_w 0.7. At higher values of a_w, the difference between total water and nonfreezable water increases greatly. The limits of accuracy inherent in the method do not permit sampling at very high moisture contents for a reasonable extrapolation to the

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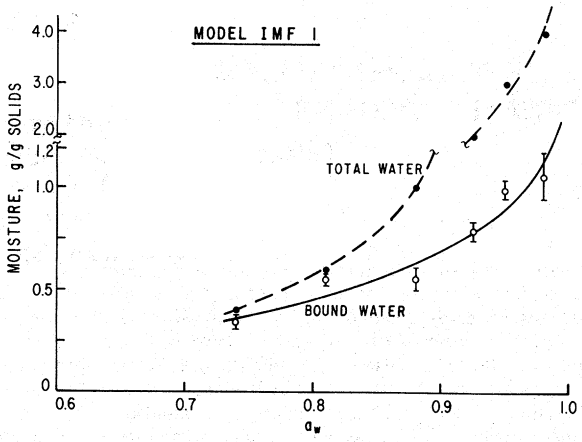

Fig. 1—Total water and bound water in IMF 1 as a function of a_w.

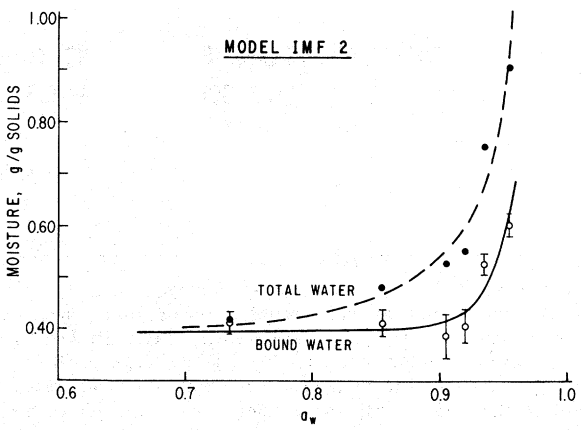

Fig. 2—Total water and bound water in IMF 2 as a function of a_w.

infinite dilution value of bound water. Below 5% total solids, the expected error in measuring bound water would be approximately the same as the magnitude of bound water itself. Nevertheless, the data do indicate a monotonic increase in bound water with increasing a_w.

Figure 2 shows the results for model IMF 2. As with IMF 1, the variation of bound water with a_w is apparent when the data are expressed as g H_2O/g solids. The limit of detection of freezable water again is at about a_w 0.7, but in this example, bound water remains fairly constant until $a_w > 0.9$. Above a_w 0.9, bound water reaches a magnitude of about 1.5 times its value at low a_w.

One might suppose that the variation in bound water in Figure 1 and Figure 2 results from freezing inhibition by solutes (Luyet and Rasmussen, 1968; Luyet, 1969), or lowering of the ΔH of fusion of ice (Haly and Snaith, 1971; Biswas et al., 1975). However, both these effects would tend to increase the apparent value of bound water at low a_w. For example, a plot of integral heat of fusion versus moisture shows that if we were to start with the model system at a_w 0.8 and add moisture to get to a_w 0.95, ΔH of the additional moisture would be nearly identical to that for bulk water. Similarly, solute concentration would decrease with the increasing moisture content and thus would contribute a lesser effect on freezing. In fact, we might conclude that bound water is overestimated at low a_w, and thus its change with a_w is at least as much as is indicated in the two figures, and perhaps more.

Figure 3 shows the bound water and total moisture isotherms for the simpler system of Na-caseinate in water. The fairly high content of salts and low-molecular weight material in this sample of commercially available caseinate provided the means for significantly varying a_w by changing only the concentration of caseinate from 5 to 60% total solids. With fewer components than in the first two systems, the bound water isotherm assumes a smoother shape which is more closely analogous to that for total moisture.

In order to isolate the effects of low- and high-molecular weight fractions of Na-caseinate on water binding, the fractions were separated by dialysis. The soluble components, which were separated by negative pressure dialysis, showed a concentration-dependent (i.e., a_w-dependent) bound water content over the range 5–25% total solids. Bound water in these redispersed samples was found to lie between 0.55 ± 0.02g H_2O/g solids at high concentration (low a_w) and 0.75 ± 0.12 g/g at low concentration (high a_w). In each of the three dispersions of isoelectric casein, a_w was > 0.99 and bound water was determined to be 0.49 ± 0.08g H_2O/g casein. This value agrees well with the results of Berlin et al. (1970), who found 0.55 g/g, and Ruegg et al. (1974), who found 0.46 g/g.

A limitation of the bound water isotherm for Na-caseinate

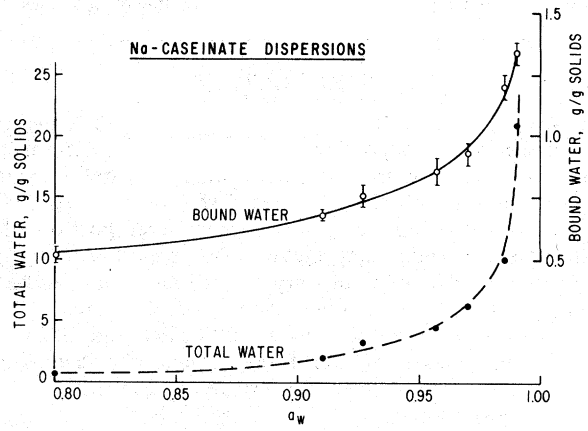

Fig. 3—Total water and bound water in dispersions of Na-caseinate as a function of a_w.

(Fig. 3) is the fact that the ratio of water to caseinate was not constant. One would prefer to measure bound water as a function of a_w at constant H_2O:casein ratio. In order to make such measurements, however, some solute must be added to the system, which may change the extent of freezing of water. In addition, the solute may bind to casein, thereby changing the solution phase concentration and possibly covering potential sites for water binding.

One useful solute for controlling a_w is urea, which, according to Lewis and Burrows (1912) and Scatchard et al. (1938), forms a nearly ideal aqueous solution up to 20 molal, i.e., Raoult's Law may be used to calculate a_w. An even greater advantage is depicted in Figure 4: the water-urea system has a eutectic point at about −12°C. The relative peak areas are dependent on concentration, as is the position of the higher temperature peak (representing water not in the eutectic mixture). Thus, there are two independent measures of urea concentration in the solution phase of a mixture containing water-urea-macromolecule. A third fortuitous advantage of urea is that the total fusion peak area equivalent to 1 mg H_2O in solution is the same, within experimental error, as for pure water. Hence, the total area of the fusion endotherm (both peaks) provides a measure of freezable H_2O.

The results of bound water measurement for casein-urea-water systems at pH 6.8 are shown in Table 1. The first item of interest is the higher value of bound water for casein in water at pH 6.8 than for isoelectric casein. The difference may

be attributed to an increase in water bonding with increasing pH as suggested by the total moisture sorption isotherms published by Ruegg and Blanc (1976). With increasing urea concentration (decreasing a_w), the amount of bound water decreases, until at 6M all the moisture in the sample is accounted for in the observed fusion peak. In other words, at constant protein to water ratio, as the concentration of urea increases, less and less water associates with the casein in a way which would make the water nonfreezable.

As mentioned above, the fusion endotherms for these systems containing water and urea indicate the actual solution phase concentration of urea. Thus, in principle one may compute the total solution phase urea content and the extent of urea binding. Unfortunately, the inherent error in such calculations is too large to permit meaningful conclusions. Representative values of urea binding are: 0.3g urea/g casein at 0.5M, 0.7 g/g at 1M, 0.9 g/g at 1.5M, and 0.4 g/g at 2M and higher concentrations (M = molal concentration). The uncertainty in each of these binding figures is about ± 0.3 g/g. Nevertheless, the total binding of water plus urea is lower at lower a_w, indicating that competition for binding sites is not the determining factor in the reduction of water binding.

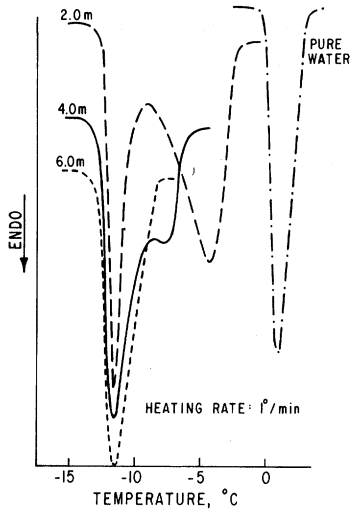

Fig. 4—DSC thermograms of frozen urea solutions. The endothermic peak at about −12° C represents the melting of the eutectic mixture.

Table 1—Bound water in casein-urea-water systems at pH 6.8

Urea conc (molal)	a_w	Bound water, g/g casein
0	>0.99	0.95 ± 0.11
0.5	0.99	1.19 ± 0.32
1.0	0.98	1.09 ± 0.34
1.5	0.97	1.00 ± 0.40
2.0	0.96	0.73 ± 0.16
3.0	0.95	0.54 ± 0.25
4.0	0.93	0.10 ± 0.25
6.0	0.90	0 ± 0.2

Table 2—Bound water in Avicel-urea-water systems

Urea conc (molal)	a_w	Bound water, g/g Avicel
0	>0.99	1.09 ± 0.11
1.0	0.98	1.00 ± 0.18
3.0	0.95	0.62 ± 0.20
6.0	0.90	0 ± 0.20

One must bear in mind that urea is known to be a disruptive or denaturing agent for proteins. Thus, the possibility exists that the apparent changes in bound water arise from denaturation, aggregation, or loss of solubility with increasing urea concentration. Nevertheless, casein is not subject to denaturation in the usual sense and it is known to be soluble in 6M urea at about neutral pH (Swaisgood, 1973). Only an aggregation-dissociation equilibrium might influence the bound water measurements, but such an effect would not account for a 100% reduction of bound water in 6M urea.

Avicel microcrystalline cellulose was selected as an additional test material representative of polysaccharides, in spite of its low solubility. The confounding effects of protein aggregation-disaggregation equilibria in urea solution were presumed to be absent in dispersions of Avicel. The experiments were analogous to those with casein-urea-water, except that the Avicel dispersions were at pH 5. The results are shown in Table 2. A urea concentration of 6 molal was sufficient to eliminate all bound water from Avicel, just as in the casein experiments.

In every model system under consideration the extent of water binding, expressed as g H_2O/g solids, decreases as a_w decreases. This relationship is obtained when a_w is controlled by varying total moisture or by adding a solute to dispersions of fixed water:macromolecule ratio. Correcting for the contribution of glycerol, sucrose, and NaCl to bound water determinations can only decrease bound water even more with decreasing a_w for IMF 1 and IMF 2. Similarly, the possibility of lower heat of fusion of ice at lower moisture contents would tend to further decrease bound water at low moisture or low a_w. The systems containing urea were studied because of the absence of these potential errors. Using urea to control a_w at constant moisture content gave results consistent with those of the variable moisture model systems.

We may conclude that for a specific system in which at least one component does bind water the extent of binding is dependent on a_w. As a_w decreases, the amount of bound water per gram of macromolecule decreases concomitantly. The data presented here show that the relationship between bound water and a_w is certainly not linear, but the data are not sufficient to assign any simple functional form to the relationship.

Biswas et al. (1975) observed a similar relationship between hydration and a_w in aqueous solutions of carbohydrates by the same DSC technique. The hydration of sucrose, β-lactose, and the maltose decreased from > 9 g/g at 1% concentration (w/w) to an assymptotic limit of > 1 g/g above 30% concentration. The authors interpreted their results in terms of an equilibrium between hydrated and unhydrated solute:

$$S + n\,H_2O \rightleftharpoons S\,(H_2O)_n$$

The law of mass action dictates that as the activity of one reactant (viz, H_2O) decreases, the equilibrium activity (and concentration) of the product species (hydrated solute) must also decrease.

Their study included several polysaccharides which, like the sugars, had concentration-dependent water binding with the greatest hydration at low concentrations. However, the concentration range was so low ($< 1\%$ total solids and $a_w > 0.99$) that the unstated confidence limits on their bound water determinations must have been large.

The results reported here support the interpretation of Biswas et al. (1975) and extend the concept to macromolecular solutes at a_w values extending into the intermediate moisture range. The data for IMF 1 and IMF 2 also suggest that water binding is an equilibrium process governed by a_w in heterogeneous mixtures as well as in aqueous solutions.

In summary, the existence of a "bound water isotherm"

suggests that one must exercise caution when attempting to interpret bound water measurements; the macromolecular concentration and the a_w of the system must be specified in order to compare results from various systems and laboratories.

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